

The assaying method of the present invention, in the case where a component in the high-density lipoprotein (HDL) is to be assayed is characterized by introducing therein sufficiently increasing the ion strength of the reaction solution, and/or activating lipoprotein lipase and/or cholesterol esterase that acts preferentially on HDL, and/or using a nonionic surfactant that has reaction selectivity to HDL and has an HLB value of 16 or more.

Further, the assaying method of the present invention in the case where the cholesterol in low-density lipoprotein (LDL) is to be assayed, provides a method comprising at first selectively subjecting a cholesterol component in an HDL fraction to an enzymatic reaction to assay or digest thereof in a first enzymatic reaction system, and then assaying the cholesterol component in the LDL fraction by an enzymatic reaction by utilizing a nonionic surfactant that has an HLB value of 11 to 13 in a second enzymatic reaction system.

Furthermore, the assaying method of the present invention provides a method comprising simultaneously or separately treating a first enzyme system and second enzyme system to have the cholesterol component in a very low-density lipoprotein (VLDL) fraction remained, and then introducing a means for decomposing the VLDL fraction to assay the cholesterol component in the VLDL fraction by an enzymatic reaction. The cholesterol in the VLDL fraction may be assayed without accompanying elimination of HDL and LDL.

The assaying method of the present invention also includes an assaying method further comprising a step for adding cholesterol

oxidase or cholesterol dehydrogenase to the above-mentioned assaying method of the present invention to digest free cholesterol.

The assaying method of the present invention described above is characterized in that the pH of the enzymatic reaction solution is within such a range that the lipoprotein does not cause agglutination nor make the reaction solution cloudy and is selected in view of an optimum pH of the enzyme that catalyzes the enzymatic reaction of the component in the lipoprotein.

10 BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a diagram illustrating the effect of addition of lipoprotein lipase (LPL; derived from *Chromobacterium viscosum*). (Experiment 1) In the figure, -●- indicates the relative reaction amount (%) of an HDL fraction, -○- indicates the relative reaction amount (%) of an LDL fraction, and -□- indicates the relative reaction amount (%) of a VLDL fraction.

Fig. 2 is a diagram illustrating the effect of addition of hydrazine. (Experiment 2) In the figure, -●- indicates the relative reaction amount (%) of an HDL fraction, -○- indicates the relative reaction amount (%) of an LDL fraction, and -□- indicates the relative reaction amount (%) of a VLDL fraction.

Fig. 3 is a diagram illustrating the effect of addition of a nonionic surfactant that has an HLB value of 17.3, Nonion K-230. (Experiment 3) In the figure, -●- indicates the relative reaction amount (%) of an HDL fraction, -○- indicates the relative reaction amount (%) of an LDL fraction, and -□- indicates the relative reaction

amount (%) of a VLDL fraction.

Fig. 4 is a diagram illustrating the effect of addition of an ion strength controlling substance for enzymatic reaction solution, nonionic surfactant NONION K-230, and selected enzyme.

5 (Experiment 4) In the figure, -●- indicates the relative reaction amount (%) of an HDL fraction, -○- indicates the relative reaction amount (%) of an LDL fraction, and -□- indicates the relative reaction amount (%) of a VLDL fraction.

10 DETAILED DESCRIPTION OF A PREFERRED EMBODIMENT OF THE INVENTION

Controlling the reactivity of an enzyme on a specific lipoprotein component by addition of an ion strength controlling substance for an enzymatic reaction solution according to the present invention, means dissolving a specific lipoprotein fraction by
15 utilizing the property that respective lipoprotein fractions, namely, HDL, LDL and VLDL, have different water solubilities and selectively carrying out the enzymatic reaction with a component in the specific fraction. As one means for achieving the object, the ion strength of a specimen is elevated. The ion strength for selectively
20 dissolving HDL can be obtained by adding, for example, a hydrazine in a concentration of about 30 mM, preferably 60 mM or more. As the hydrazine, hydrazine groups as well as salts, hydrates and solvates thereof, that are selected to be used based on the selective solubility of HDL as an index. Similarly, NaCl, urea, guanidines,
25 semicarbazides, and the like may also be used. The compounds which proceeds the ion strength may be used singly or a plurality in